2018-07

Differences in leaf thermoregulation and water use strategies between three co-occurring Atlantic forest tree species

Fauset, S::0000-0003-4246-1828

10.1111/pce.13208
Plant, Cell and Environment
Wiley

Differences in leaf thermoregulation and water-use strategies between three co-occurring Atlantic forest tree species

Running title: *Leaf energy balance of Atlantic forest trees*

Authors: Sophie Fauset[1], Helber C. Freitas[2], David R. Galbraith[1], Martin J.P. Sullivan[1], Marcos P.M. Aidar[3], Carlos A. Joly[4], Oliver L. Phillips[1], Simone A. Vieira[5], Manuel U. Gloor[1].

[1] School of Geography, University of Leeds, Leeds, LS2 9JT, UK.

[2] Departamento de Física, Faculdade de Ciências, Universidade Estadual Paulista, Av. Eng. Luiz Edmundo Carrijo Coube, 14-01, Bauru, São Paulo, 17033-360, Brazil.

[3] Instituto de Botânica de São Paulo, Avenida Miguel Stéfano, São Paulo, 04301-902, Brazil.

[4] Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Rua Monteiro Lobato, Cidade Universitâria, Campinas, São Paulo, 13083-862, Brazil.

[5] Núcleo de Estudos e Pesquisas Ambientais, Universidade Estadual de Campinas, Rua dos Flamboyants, 155, Campinas, São Paulo, 13083-867 Brazil.

Corresponding Author: Sophie Fauset, s.fauset@leeds.ac.uk

Summary Statement

In the first study of leaf energy balance in tropical montane forests, we observed current leaf temperature patterns in the Atlantic forest, Brazil, and assessed whether

and why patterns may vary among species. We found large leaf-to-air temperature differences that were influenced strongly by radiation, and differences in leaf temperature between two species due to variation in leaf width and stomatal conductance. We highlight the importance of leaf functional traits for leaf thermoregulation, and also note that the high radiation levels which occur in montane forests may exacerbate the threat from increasing air temperatures.

Abstract

Given anticipated climate changes, it is crucial to understand controls on leaf temperatures including variation between species in diverse ecosystems. In the first study of leaf energy balance in tropical montane forests, we observed current leaf temperature patterns on three tree species in the Atlantic forest, Brazil, over a 10-day period, and assessed whether and why patterns may vary among species. We found large leaf-to-air temperature differences (maximum 18.3°C) and high leaf temperatures (over 35°C) despite much lower air temperatures (maximum 22°C). Leaf-to-air temperature differences were influenced strongly by radiation, while leaf temperatures were also influenced by air temperature. Leaf energy balance modelling informed by our measurements showed that observed differences in leaf temperature between two species were due to variation in leaf width and stomatal conductance. The results suggest a trade-off between water-use and leaf thermoregulation; *Miconia cabussu* has more conservative water-use compared to *Alchornea triplinervia* due to lower transpiration under high vapour pressure deficit, with the consequence of higher leaf temperatures under thermal stress conditions. We highlight the importance of leaf functional traits for leaf thermoregulation, and also note that the high radiation levels

which occur in montane forests may exacerbate the threat from increasing air temperatures.

Key-words: leaf temperature, tropical forest, montane, leaf width, stomatal conductance, transpiration, radiation, functional traits, boundary layer, energy balance.

Introduction

The surface temperature of the Earth is increasing (Rahmstorf, Foster, & Cahill, 2017) and set to continue increasing into the future (Collins *et al*., 2013). The majority of tropical forests show a trend of increasing air temperature over the past 35 years which is particularly strong in South America with recent increases up to 0.5°C per decade (Supplementary Figure. S1). Temperature extremes are also increasing and are predicted to increase in the coming century (Coumou & Robinson, 2013). Whilst it is known that temperature influences plant functioning, the response of plants to increasing temperature and variation between species is a major uncertainty (Teskey *et al*., 2015). Tropical forests are particularly important in this regard as they are a considerable store of terrestrial carbon (Pan *et al*., 2011), potentially already function near their maximum temperature (Doughty & Goulden, 2008) and given their location cannot be replaced by species from lower latitudes. The biodiversity of tropical montane forests, which house many endemic species with restricted ranges, may be at particular risk from higher temperatures due to limits on upslope migration, especially for tree species in forests occurring on mountaintops (Phillips, 1997). Modelling studies suggest increasing temperatures are also likely to have a negative effect on

tropical forest vegetation carbon, however the extent of projected impact varies greatly between different models (Galbraith *et al*., 2010; Huntingford *et al*., 2013) as do the physiological mechanisms behind the declines (Galbraith *et al*., 2010).

Temperature can impact plant physiology both directly, by influencing rates of photosynthesis and respiration, and indirectly by altering the ambient vapour pressure deficit (D) (Lloyd & Farquhar, 2008). D increases with an increase in air temperature (T_A) if relative humidity (h) stays constant, and stomatal conductance (g_s) typically declines with increasing D (Leuning, 1995) to avoid excessive water loss. The reduction in g_s with D has the consequence of reduced CO_2 concentration within the leaf. The photosynthetic optima of plants are typically near the mean maximum ambient temperature, showing the acclimation of plants to their environment (Slot & Winter, 2017). The temperature of the leaf tissue itself is relevant temperature for the control of leaf physiological processes, rather than T_A. High leaf temperatures (T_L) can induce damage to photosynthetic machinery; above *c*. 35°C thylakoid membranes have been observed to structurally change (Gounaris, Brain, Quinn, & Williams, 1983; Gounaris, Brain, Quinn, & Williams, 1984) and above 40°C photosystem II (PSII) may become deactivated and the electron transport rate reduced (Allakhverdiev *et al*., 2008). Chlorophyll fluorescence parameters to assess heat tolerance of PSII show critical temperature thresholds in the region of 45 – 60 °C, with significant variation between species (O'Sullivan *et al*., 2017; Sastry & Barua, 2017). Irreversible thermal damage to photosynthetic machinery has been observed to occur at 52 °C in a tropical species (Krause *et al*., 2010).

100 Extremes of microclimate can lead to leaf temperatures that are markedly different
101 from T_A. For example, leaf temperatures up to 10 °C above air temperatures when
102 leaves were brightly lit have been observed in the Amazon (Doughty & Goulden
103 2008) and in Panama (Rey-Sanchez, Slot, Posada, & Kitajima, 2016), and Slot,
104 Garcia, & Winter, (2016) found leaf temperatures of a *Ficus insipida* regularly
105 exceeded 40°C and even reached 48 °C during a three week period in Panama. Yet,
106 despite these striking patterns of leaf temperatures, and the on-going and anticipated
107 increases in air temperatures, there are few datasets examining fluctuations of leaf
108 temperatures *in situ* in tropical forests, and, to our knowledge, none in tropical
109 montane forests.
110
111 Leaf energy balance theory can be used to address the drivers of T_L in a mechanistic
112 approach (e.g. Michaletz *et al*. 2016). Developed from the Penman energy balance
113 approach to evapotranspiration (Penman, 1948), the leaf energy balance equation (see
114 Materials and Methods, equation 2) estimates the leaf-to-air temperature difference
115 (ΔT) for given microclimatic and leaf-specific variables (Jones, 1992). The leaf
116 energy balance shows that ΔT is dependent on the net energy provided (or lost) by
117 radiation and the energy lost through transpiration. The effects of these fluxes on ΔT
118 depend on leaf shape and physiology through the boundary layer and stomatal
119 resistances to water transport. Stomatal resistance is dependent on stomatal activity
120 and boundary layer resistance increases with leaf width (see Materials and Methods).
121 Hence, whilst ΔT is strongly influenced by microclimatic conditions (in particular
122 radiation and D), leaf traits (width and stomatal conductance) can also play a role in
123 regulation of leaf temperature. In addition, leaves can alter their physical position

124 through changes in angle and/or orientation to increase or decrease the amount of
125 radiation received.
126
127 Leaf structural traits (leaf mass per area (LMA) and leaf dry matter content (LDMC))
128 and stomatal conductance (g_s) influence the time required for leaf temperature to
129 change following a change in the environment (the thermal time constant (τ), Jones,
130 1992). Leaves with a long τ will show smaller temperature changes in a fluctuating
131 environment, maintaining the leaf temperature closer to the mean air temperature than
132 a leaf with a small τ which will track fluctuation in air temperature (Michaletz *et al*.,
133 2015).
134
135 Given the diversity of leaf structures and physiology observed within and among
136 tropical forest species (e.g. variation in leaf(let) area over 5 orders of magnitude for a
137 large sample of tropical species, Wright *et al*., 2017), it is possible that there will be
138 diversity in leaf strategies with regard to temperature (Michaletz *et al*., 2015). This
139 means that the impacts of potential future environmental changes may vary between
140 species even within a single biome. Future combined atmospheric changes of
141 increasing CO_2 and increasing T_A could be particularly important for T_L, as plants tend
142 to respond to increasing CO_2 by reducing g_s (Way, Oren, & Kroner, 2015). Reducing
143 g_s decreases water use, but also has the consequence of increasing leaf temperature
144 (Drake, Gonzàlez-Meler, & Long, 1997; Barker *et al*., 2005) and can lead to
145 premature leaf senescence under heat-wave conditions (Warren, Norby, &
146 Wullschleger, 2011). Increases in T_A could be particularly important under fluctuating
147 and extreme conditions (e.g. heat-waves), increasing the occurrence of leaves
148 reaching or exceeding threshold temperatures resulting in leaf damage.

We present an observational study of leaf temperatures in a highly threatened tropical forest region – the Atlantic forest, among the most diverse and threatened of biodiversity hotspots (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000; Colombo & Joly, 2010). Our mountaintop study site is home to many endemic species. Humans have exploited the Atlantic forest for 500 years resulting in a highly fragmented landscape (Joly, Metzger, & Tabarelli, 2014) that reduces possibilities for species migration. Hence, a greater understanding of forests in this region is of great interest given their high threat level. We focus here on determining and understanding interspecific differences in leaf temperatures caused by differences in leaf traits. Our approach aims to begin to reveal whether or not trees are likely to be able to cope with future conditions, and the extent to which species identity is likely to be important. This is a step towards an understanding of the resilience of tropical trees and is part of a broader effort to assess the effects of stressors on remaining Atlantic forests and their ability to recover.

We used a narrow canopy tower to access leaves of three trees each of different species (*Alchornea triplinervia* (Spreng.) Mull. Arg. (Euphorbiaceae), *Miconia cabussu* Hoehne (Melastomataceae) and *Guapira opposita* (Vell.) Reitz (Nyctaginaceae), hereafter referred to by genus only). We monitored leaf temperature and microclimate relevant to leaf energy balance over a period of 10 days, and quantified the stomatal behaviour and structural leaf traits of the sample trees. With this dataset we aim to answer the following questions:

i) What are the current patterns of leaf temperature of the Atlantic forest species *Alchornea*, *Miconia* and *Guapira* under fluctuating microclimatic conditions?

ii) Are there differences in leaf thermoregulation between the species?

iii) To what extent do leaf traits (width, stomatal conductance) and microclimate (radiation, T_A, D, wind speed) determine leaf temperatures?

Materials and Methods

Study Site

The field study was carried out in the Serra do Mar State Park, São Paulo state, Brazil. The park is home to the largest contiguous patch of Atlantic forest remaining, running along a steep coastal mountain range. The study site (23.3254 S, 45.0938 W) is located within a 1 ha permanent plot at 1000 m elevation. The vegetation is mid-successional secondary forest, regenerating from clear felling for charcoal before the establishment of the park in 1977 (Marchiori, Rocha, Tamashiro, & Aidar, 2016). The forest is classified as montane moist dense forest (Vieira *et al*., 2011), mean annual precipitation is 2300 mm with a dry season in July and August, mean annual temperature is 17°C (Joly *et al*., 2012) and fog occurs frequently (Rosado, Oliveira, & Aidar, 2010). Canopy height of emergent trees reaches 30 m. Data collection was carried out between 1 – 10 October 2016.

Microclimate measurements

A narrow 27 m high tower was used for access to the canopy and microclimate measurements (T_A, photosynthetically active radiation (PAR), relative humidity (h) and wind speed (U)) were collected to detail the microclimate vertical profile (Fig. 1). As the tower is just 30 cm wide and tree branches are within arms reach of the tower (see Supplementary Figure S2d), we consider that the presence of the tower likely has

only minimal influence on the microclimate of the sampled leaves. From 18 m above the ground, at the height of the highest leaves adjacent to the tower, 16 PAR sensors were suspended from the tower at *c*. 1 m intervals, with an additional sensor positioned at 25 m above the ground. Sensors were made following Fielder & Comeau (2000) using gallium arsine phosphide (GaAsP) photodiodes (G1118, Hamamatsu, Japan) and calibrated against a LI-COR 190 quantum sensor (LI-COR Inc., Nebraska, USA). PAR sensors were positioned on plastic supports in the horizontal plane. In addition, 7 thermistors (107, Campbell Scientific, Utah, USA) to measure T_A were deployed in radiation screens spread through the vertical profile (heights 1.5, 5, 7.5, 10, 12.5, 15 and 18 m, Fig. 2). PAR and T_A data were measured and recorded at 10 s intervals using two CR800 data loggers with AM 16/32 multiplexers (Campbell Scientific, Utah, USA). Four data-logging *h* sensors (RHT10, Extech, Massachusetts, USA) measured and recorded at 1 min intervals at heights 2, 8, 12.5 and 18 m. Four sonic anemometers (Sonicwind) measured *U* every 0.5 s at heights of 1.5, 6.5, 11.5 and 25 m, and 10 s averages were produced for each height. *U* for leaves positioned above 11.5 m height was linearly interpolated between the 25 m and 11.5 m measurement.

Vapour pressure deficit (*D*) was calculated from T_A and *h* (Campbell & Norman, 1998),

$$D = e_{sat} \cdot (1 - h) \quad (1)$$

$$e_{sat} = a \cdot exp \cdot \left(\frac{bT_A}{T_A + c}\right) \quad (2)$$

where T_A is in °C, h is relative humidity (as a proportion), e_{sat} is saturating vapour pressure in kPa and a, b and c are constants (a = 0.611, b = 17.502, c = 240.97).

Due to a sensor fault, h was available only from 5 – 10 October 2016. To estimate D within the profile for measurement days prior to this, we estimated h within the profile based on the observed relationship between T_A and h at the four measurement heights (R^2 0.76 – 0.87) from the available data collected over six days.

Sampled species

Both *Alchornea* and *Miconia* are overstorey species, while *Guapira* is found in the subcanopy (Guilherme, Morellato, & Assis, 2004), and the species are ranked second, fifth and sixth, respectively, in terms of abundance in the plot (Marchiori *et al*., 2016). All species are early successional (Marchiori *et al*., 2016) and are found in nearby old-growth forest. *Alchornea* and *Guapira* are present at lower elevations in the park (Joly *et al*., 2012; Rosado, Oliveira, Joly, Aidar, & Burgess, 2012). The species cover a range of leaf sizes; *Guapira* has the smallest leaves (notophyll), followed by *Alchornea* (mesophyll), with the largest leaves for *Miconia* (platyphyll).

Leaf temperature measurements

To measure leaf-to-air temperature differences (ΔT, also called 'leaf temperature excess' in the literature) we followed the two-junction thermocouple design of Singsaas & Sharkey (1998). This approach has the advantage of more accurately measuring ΔT than performing measurements of absolute T_L and T_A separately. Two long (15 cm length) constantan fine wires (0.07 mm diameter) were soldered to either end of a short (3 cm length) copper fine wire (0.07 mm diameter). These

248 thermocouples produce a voltage proportional to the temperature difference between
249 the junctions. Thermocouples were individually calibrated by inserting one junction
250 into sand in a temperature controlled dry bath (TCBD-02, Cleaver Scientific, UK)
251 with the second junction in the air *c*. 2 cm above the bath. The temperature of the bath
252 and the air were each measured with two thermistors. Four different temperature
253 differences between the bath and air were produced (~0, 5, 10 and 12°C). Data for the
254 calibration were selected during periods with a constant dry bath temperature (i.e.
255 excluding periods when the bath was heating up or cooling down).
256
257 Between 30 September and 3 October 2016 thermocouples were installed on 10
258 *Alchornea* leaves, 9 *Miconia* leaves and 4 *Guapira* leaves. Selected leaves were fully
259 expanded and mature, but not senescent, within reach from the canopy tower, and
260 spread through the vertical profile (Fig. 1). The thermocouple junction to measure leaf
261 temperature was secured to the abaxial mesophyll surface (avoiding any large veins)
262 near to the midrib using surgical tape (Transpore, 3M, Minnesota, USA). The second
263 junction was suspended in the air *c*. 2 cm below the leaf. Additional cabling was
264 cable-tied to a twig near to the leaf (or the petiole in the case of *Miconia*) and to the
265 tower. This attachment procedure enabled the majority of thermocouples to remain
266 attached to leaves during wind and rain (see Supplementary Figure S2 for
267 photographs of the equipment installation). The petioles of two leaves, both of
268 *Miconia*, snapped during the monitoring period. Supplementary Table S1 gives details
269 of the sampled leaves. ΔT was measured and recorded at 10 s intervals using a CR800
270 data logger with AM 16/32 multiplexer (Campbell Scientific, Utah, USA) until 11
271 October 2016.
272

Spot measurements

In addition to continuous measurements of ΔT from the thermocouples, instantaneous spot measurements were made of leaf temperature using an infra-red (IR) thermometer (62MAX+, Fluke, Washington, USA) on the adaxial and abaxial leaf surfaces, PAR at the adaxial leaf surface (accounting for leaf angle and orientation) with a quantum sensor (LightScout, Spectrum Technologies, Illinois, USA) and g_s using a porometer (SC-1, Decagon Devices, Washington, USA). These measurements were made in order to i) validate the thermocouple data against an independent T_L measurement, ii) compare PAR received at the leaf surface with that measured from the tower, and iii) investigate variation in g_s between species and over time. Spot measurements were collected during daylight hours throughout the day on 6 days between 4 – 10 October 2016. Stomatal conductance measurements could only be performed when the leaf surface was dry. Hence, fewer spot measurements of g_s were collected (213 in total, on average *c*. 2 measurements per leaf per day) compared with leaf temperature on adaxial surface (785 in total, on average *c*. 6 measurements per leaf per day), leaf temperature on abaxial surface (398 in total, on average *c*. 3 measurements per leaf per day) and PAR (350 in total, on average *c*. 3 measurements per leaf per day). Measurements of g_s with the SC-1 porometer are completed in 30 s, and as the response of stomata to a change in the environment is on the order of minutes (e.g. Vialet-Chabrand et al., 2017) we assume that the leaf g_s will not have changed due to the altered microclimate of the porometer chamber within the measurement interval. Leaf angle (angle to the horizontal) was measured sporadically (minimum 5 measurements per leaf) using a clinometer (Suunto, Finland). No spot measurements were carried out during the night.

298 *Leaf trait measurements*

299 All sampled leaves were collected and stored in moist plastic bags for 24 hours before
300 fully rehydrating and measuring structural traits in the lab at the Instituto de Botânica,
301 São Paulo. Measurements were performed of leaf thickness (mm) with a digital
302 calliper, leaf area (cm^2) with leaf area meter (LI-3100, LI-COR, Nebraska, USA), leaf
303 mass (g), leaf width (cm), and leaf length (cm). Petioles were removed before
304 measurements. Subsequently, leaves were dried at 70 °C, and dry weight measured.
305 These measurements were used to calculate leaf mass per area (LMA, $g\ m^{-2}$) and leaf
306 dry matter content (LDMC, $g\ g^{-1}$). For *Guapira* the sample size for leaf traits was six
307 (rather than four as for leaf temperature).

308

309 *Leaf Energy Balance*

310 With input of measured microclimate, stomatal conductance and leaf width the leaf
311 energy balance (equation 3, Jones, 1992) can be estimated to predict the leaf-to-air
312 temperature difference (ΔT_e, °C). It is important to note that the leaf energy balance
313 assumes no leaf heat storage and that the leaf energy balance is considered to be in a
314 steady state. ΔT_e was estimated from spot measurements to test if ΔT_e matched
315 observations of ΔT when leaf surface PAR and g_s were directly measured, and from
316 the continuous microclimate data with g_s estimated from the observed species-specific
317 relationships between g_s and D in order to assess the influence of microclimate and
318 leaf specific variables on leaf temperatures using a large dataset. As the *Guapira*
319 leaves were not exposed to a large range of microclimates due their position in the
320 understorey, we only consider ΔT_e of *Miconia* and *Alchornea* in the latter analysis.

321

322 $$\Delta T_e = T_L - T_A = \frac{r_{b,HR}(r_{b,W}+r_{l,W})\gamma R_{ni}}{\rho_a c_{pa}[\gamma(r_{b,W}+r_{l,W})+sr_{b,HR}]} - \frac{r_{b,HR}D}{\gamma(r_{b,W}+r_{l,W})+sr_{b,HR}} \quad (3)$$

where T_L and T_A are the leaf and air temperatures respectively (°C), R_{ni} is the net isotropic radiation (W m^{-2}, assuming the sky temperature is equal to T_A measured at the nearest T_A sensor to the leaf and sky emissivity of 0.97), γ is the psychrometric constant (Pa K^{-1}), $r_{b,HR}$ is the boundary layer resistance to heat and radiation and $r_{b,W}$ and $r_{l,W}$ are the boundary layer and leaf resistances to water respectively (all resistances in s m^{-1}), ρ_a is the density of dry air (kg m^{-3}), c_{pa} is the specific heat capacity of dry air (1012 J kg^{-1} K^{-1}), s is the slope of relationship between temperature and saturated vapour pressure evaluated at T_A, and D is the vapour pressure deficit (Pa).

Leaf traits (g_s and leaf width) are included in equation 3 through the leaf and boundary layer resistances. Leaf resistance to water, $r_{l,W}$, is the inverse of g_s, Boundary layer conductance to heat or water, $g_{b,H}$, which is included in the determination of both $r_{b,HR}$ and $r_{b,W}$ which are both used in equation 3, is dependent on leaf width (W, m) and wind speed (U, m s^{-1})

$$g_{b,H} = 0.0105\left({}^{U}/_{W}\right)^{0.5} \quad (4)$$

Further details on the estimation of leaf energy balance are given in Appendix 1.

The thermal time constant (τ, s) was defined following Michaletz *et al*. (2016) as

$$\tau = \varphi \cdot LMA \cdot \left(\frac{c_{pw}}{LDMC \cdot H} + \frac{c_{pd} - c_{pw}}{H}\right) \quad (5)$$

where φ, the ratio of projected to total leaf area, is 0.5 for flat leaves, LMA is in units kg m^{-2}, c_{pw} is the specific heat capacity of water (4181 J kg^{-1} K^{-1}), c_{pd} is the specific heat capacity of dry leaf matter (J kg^{-1} K^{-1}). c_{pd} varies by species and here we use 2814 J kg^{-1} K^{-1}, the mean of seven tropical tree species from Jayalakshmy & Philip, (2010). H is a heat transfer coefficient (W m^{-2} K^{-1}) accounting for convection, radiation and transpiration (Michaletz *et al*., 2016)

$$H = \rho_a c_{pa}\left(g_{b,H} + g_{b,R} + g_s s/\gamma\right) \quad (6)$$

where $g_{b,H}$ and $g_{b,R}$ are the boundary layer conductance to heat and radiation respectively (both m s^{-1}, see Appendix 1). τ varies over time due to its dependence on g_s and boundary layer resistance, and was estimated from spot measurements.

Leaf boundary layer resistance

Initial estimations of the leaf energy balance using equation 3 showed that when ΔT_e was evaluated at low wind speeds (< 0.5 m s^{-1}) the values were overestimated compared to the observed ΔT. Using equations 4 and S5 to estimate the boundary layer resistance to water ($r_{b,W}$), there is a steep increase in $r_{b,W}$ below wind speeds of 0.5 m s^{-1} (Supplementary Figure S3). To test if these high resistances were supported by the data, we solved the leaf energy balance equation for $r_{b,W}$ and estimated $r_{b,W}$ using the observations of ΔT (see Appendix 2). Plotted against wind speed, the estimated $r_{b,W}$ was lower than predicted by equations 4 and S5 at low wind speeds (Supplementary Figure S3). Hence, we re-parameterised constants from equation 4 using the $r_{b,W}$ estimated from the leaf energy balance and observed wind speed and leaf width (see Appendix 2). Parameter estimation was performed separately for

373 *Miconia* and *Alchornea* (there was not sufficient data for parameter estimation of
374 *Guapira*) using non-linear least squares (R function nls).
375
376 In order to have accurate estimates of $r_{b,W}$ from the energy balance, it is essential that
377 all microclimate inputs are correct. PAR was measured at various points from the
378 tower (Fig. 1). Examination of the spot measurement data showed that PAR measured
379 by the nearest sensor suspended from the tower (maximum 1 m distance from leaf)
380 occasionally strongly overestimated or underestimated leaf surface PAR
381 (Supplementary Figure S4) as they are not measured at precisely the same location,
382 angle or orientation, and PAR shows high spatial variability. To select only data
383 where PAR measured from the tower appropriately represented PAR at the leaf
384 surface, the daytime data was split into twenty minute periods and ΔT_e estimated for
385 every 10 s datapoint. Linear regression was then used to identify periods where ΔT_e
386 matched measured ΔT, selecting only periods where the slope of the relationship
387 between ΔT_e and ΔT was 1 ± 0.3 and the intercept was ± 2 °C. Based on this selection
388 procedure we identified 20 % of the dataset (*c*. 150,000 data points) considered to
389 have representative PAR measurements. This approach does not entirely eliminate
390 noise from the dataset as within the 20 min period there can still be some erroneous
391 data-points.
392
393 *Data Analysis*
394 Linear mixed effects models with leaf as a random factor were used for all statistical
395 analyses including repeated measurements of the same leaf using the R package nlme
396 (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2017). R^2 for mixed effects
397 models are given using either the marginal-pseudo-R^2 that accounts for fixed factors

only or conditional pseudo-R^2 (Nakagawa & Schielzeth, 2013). The marginal-pseudo-R^2 is used unless otherwise stated, and R^2 values were calculated using the function provided in the R package MuMIn (Bartoń, 2016). Statistical analyses comparing between species using single values for each leaf used ANOVA for three species comparisons and t-test for two species comparisons.

Relationships between g_s and D were analysed for each species using a linear mixed effects model with leaf as a random factor. The relationships produced were used to estimate a time-series of g_s for each leaf based on D. The intercept of the $g_s \sim D$ relationship was thus leaf specific, and the slope species specific.

To compare leaf temperatures under comparable microclimate conditions data was first selected for 20 min periods where ΔT_e matched measured ΔT to ensure microclimate variables are representative of the leaf surface, as for *Leaf boundary layer resistance* (see above) but using the species-level parameterization of $r_{b,W}$ to estimate ΔT_e. The selected dataset was then subsetted according the microclimate (PAR, T_A and U) for each leaf. We produced subsets of ΔT under low PAR and T_A (PAR 50-150 μmol m^{-2} s^{-1}, T_A 13-15 °C), medium PAR and T_A (PAR 50-150 μmol m^{-2} s^{-1}, T_A 13-15 °C), high PAR and T_A (PAR 1000-1300 μmol m^{-2} s^{-1}, T_a 18-20 °C), and very high PAR (PAR 1600-1900 μmol m^{-2} s^{-1}, T_A 18-20 °C), all at wind speed 0.5 – 1.5 m s^{-1}. Differences in ΔT between species for each microclimate were evaluated with linear mixed effects models with leaf as a random factor.

Results

Validation of thermocouple data

T_L based on ΔT measured with thermocouples and T_A measured with thermistors ($T_L = \Delta T + T_A$) was highly correlated with T_L as measured by the IR thermometer (Pearson's correlation coefficient for each leaf 0.60 - 0.99, where T_L was measured with IR thermometer on the abaxial leaf surface). The slopes of linear regression lines forced through zero were significantly different from 1 for only three leaves, where the thermocouples slightly underestimated T_L by up to 9 % (Supplementary Figure S5). Overall, the close agreement between the two measurement methods gives confidence in the thermocouple data.

Microclimate during the monitoring period

Microclimate during the monitoring period is shown in Supplementary Figure S6 and for a single sunny day in Fig. 2. The first seven days (1 – 7 Oct) were predominately overcast with low PAR, high *h* and low *D,* with some sunny periods on 6 and 7 Oct. Subsequently, two days (8 and 9 Oct) had longer bright periods. The final day of data collection (10 Oct) was again overcast. Throughout the period lower canopy levels received substantially less PAR and experienced lower *D*. However, on sunny days, high PAR levels and higher *D* extended throughout the vertical profile (e.g. 8 October 2016, Fig. 2). Mean daytime T_A at the top of the canopy was 15.0°C, with a maximum T_A of 22.1°C recorded on 8 October 2016. Mean nighttime T_A was 12.4°C, and was lowest preceding sunny days. Mean windspeed above the canopy (at 25 m) was 1.0 ± 0.7 m s^{-1}, and 0.26 ± 0.18 m s^{-1} within the canopy (averaged over all sample heights 1.5, 6.5 and 11.5 m).

Patterns of leaf temperature

448 Leaves occasionally reached much higher temperatures than the ambient air, over 10
449 °C above T_A. The maximum T_L for each leaf observed over the monitoring period
450 ranged from 22.5-37.2 °C and was above 35 °C for 5 of the 23 leaves. *Miconia* leaves
451 had significantly higher maximum temperatures than *Alchornea* leaves (Table 1).
452 Daytime mean T_L showed less variation than maximum T_L between leaves and species
453 (Table 1, Supplementary Figure S7). Leaves of *Guapira*, which were all at the bottom
454 of the canopy, had lower maximum and mean T_L (not tested for significance due to
455 low sample size). Distributions of T_L show positive skew (Supplementary Figure S7)
456 which was significantly higher for leaves of *Miconia* than *Alchornea* (Table 1),
457 showing that they reached high temperatures more often than *Alchornea*. During
458 daylight hours ΔT was positive for leaves of *Alchornea* and *Miconia*, and was close to
459 zero for all *Guapira* leaves (Supplementary Figure S8, Table 1). The maximum ΔT
460 observed was 18.3 °C, again recorded from *Miconia* leaf M1. As for T_L the
461 distributions of ΔT were positively skewed, with significantly higher skewness for
462 *Miconia* than *Alchornea* (Table 1). Minimum daytime ΔT was significantly lower for
463 *Alchornea* than *Miconia* leaves (Table 1). Night-time ΔT was typically negative but
464 close to zero, and was significantly lower for *Alchornea* than *Miconia* (Table 1).
465
466 T_A set a rough minimum bound on T_L (Fig. 3), with many excursions above T_A due to
467 high radiation (see section *Leaf Energy Balance*) and a small number of excursions
468 below T_A, likely occurring when leaf surfaces were wet during/after rain or in fog. T_L
469 excursions above T_A occurred more often for leaves positioned higher in the canopy.
470
471 *Leaf temperatures under specific microclimates*

We compared leaf temperatures under specific microclimatic conditions. Under low light and temperature conditions leaves of *Guapira* were significantly colder than those of *Alchornea* and *Miconia* (Fig. 4a); there was no significant difference in ΔT between the latter two species. Under medium light and temperature conditions there again was no significant difference in ΔT between *Alchornea* and *Miconia* (Fig. 4b); *Guapira* leaves did not experience these or brighter light conditions due to their position in the understory. Under high light and temperature conditions ΔT was significantly higher for *Miconia* than *Alchornea* (Fig. 4c). Under the highest light conditions analysed ΔT was again higher for *Miconia* than *Alchornea*, however the difference was not quite significant (Fig. 4d).

Thermal trait variation between species

Stomatal conductance (g_s) significantly declined with increasing D, and the relation varied significantly between species (Fig. 5, Table 2). At low D, g_s was highest for *Miconia* and lowest for *Guapira*. *Miconia* showed a significantly stronger negative relationship between g_s and D than *Alchornea*, hence at higher values of D, *Miconia* leaves had lower g_s. Conditional R^2 for the overall mixed model including the random factor for leaf was 0.49.

Structural leaf traits with importance for thermoregulation also varied between species (Table S1, Fig. 6). *Miconia* leaves were significantly wider, larger and had higher LMA than both *Alchornea* and *Guapira* (Fig. 6a-c). LDMC significantly differed between all species and was highest for *Miconia* (0.42 ± 0.013 g g^{-1}) followed by *Alchornea* (0.37 ± 0.016 g g^{-1}) and finally *Guapira* (0.20 ± 0.022 g g^{-1}) (ANOVA $F = 38.8$, $p < 0.0001$, and Tukey post-hoc test).

497

498 The thermal time constant (τ) ranged over two orders of magnitude from 9 - 350 s
499 (Fig. 7a) and varied significantly between species (linear mixed effects model, F =
500 48.1, df = 20, p < 0.0001). τ for *Guapira* were significantly longer and more varied
501 (mean ± sd 155.4 ± 84.0) than both *Alchornea* (mean ± sd 276.5 ± 11.1) and *Miconia*
502 (mean ± sd 46.4 ± 14.4). τ decreased with increasing g_s and was particularly high
503 under very low g_s (Fig. 7b). For a given g_s, τ increased in the order *Alchornea* <
504 *Miconia* < *Guapira* (Figure 7b). These differences were driven by the leaf structural
505 traits LMA and LDMC (Supplementary Figure S9). When estimated using a fixed
506 LMA value the differences between *Alchornea* and *Miconia* are lost (Supplementary
507 Figure S9b) showing that the higher LMA of *Miconia* increases τ. When estimated
508 using a fixed LDMC value the *Guapira* values collapse into line with *Alchornea* (the
509 two species have similar LMA) (Supplementary Figure S9c) showing that the lower
510 LDMC of *Guapira* increases τ.
511
512 *Leaf energy balance and drivers of ΔT and T_L*
513 Leaf-to-air temperature difference estimated from leaf energy balance (ΔT_e) using the
514 spot measurements matched observed ΔT well, but with some underestimation at
515 higher ΔT (Fig. 8), showing that our data adequately parameterized the leaf energy
516 balance for instances when leaf surface PAR and g_s were measured. To investigate the
517 drivers of ΔT with the larger dataset of continuous ΔT and microclimate
518 measurements, the dataset was restricted to periods where predicted ΔT_e matched
519 observed ΔT, as for the analysis of ΔT under specific microclimate conditions. This is
520 to ensure we are using appropriate values of PAR which was not measured at the leaf
521 surface in the continuous dataset. Both observed ΔT and ΔT_e increase with PAR (Fig.

9), a pattern repeated when T_L and T_{Le} (leaf temperature estimated from energy balance) were analysed (Supplementary Figure S13). The slope of the relationship between leaf temperature variables and PAR were different between *Miconia* and *Alchornea*, where *Miconia* has higher ΔT and T_L for a given PAR (Fig. 10, Supplementary Figure S13). Whilst the absolute values of ΔT_e and T_{Le} are somewhat higher than the observations, the differences between the species are maintained in the energy balance estimations. Relationships between ΔT and ΔT_e and other microclimate variables (T_A, D, U) were much weaker than for PAR with all R^2 values below 0.3 (Supplementary Figures S10-S12), while T_L and T_{Le} were strongly related to T_A and D with R^2 values above 0.7 (Fig. S14-S16).

To determine what causes the differences between ΔT of *Miconia* and *Alchornea*, we applied traits (leaf width, stomatal conductance strategy) of *Miconia* sequentially to *Alchornea* and re-estimated ΔT_e using the observed microclimate data. As shown in Supplementary Figure 17, applying the larger leaf width of *Miconia* acts to increase the *Alchornea* ΔT_e for a given PAR, almost to the extent that it matches the high ΔT_e of *Miconia*. If the higher intercept of the g_s~D relationship for *Miconia* is applied, the *Alchornea* ΔT_e for a given PAR decreases. In contrast, if the steeper g_s~D slope for *Miconia* is applied, the *Alchornea* ΔT_e for a given PAR increases. The effect is not as strong as the effect of leaf width (Supplementary Figure 17). Applying both the *Miconia* intercept and slope results in an intermediate *Alchornea* ΔT_e for a given PAR, slightly higher than for *Alchornea* with its original parameterization. If all *Miconia* traits are applied (leaf width and stomatal conductance strategy) *Alchornea* ΔT_e for a given PAR increases to a greater extent than for any trait alone, and even exceeds the ΔT_e of *Miconia*. This is likely due to the higher D that the highest six

547 *Alchornea* leaves are exposed to due to their position above the *Miconia* leaves (Fig.
548 1).
549
550 **Discussion**
551
552 *Current leaf temperature patterns and the role of microclimate*
553
554 Despite the low T_A (maximum 22 °C) occurring during the study period we observed
555 leaf temperatures over 30 °C. Whilst few datasets are available on field-measured leaf
556 temperatures of tropical trees, the maximum ΔT we observed (18.3 °C) is somewhat
557 higher than those previously reported (e.g. 13.9 °C for a range of Proteaceae species
558 in Australia (Leigh, Sevanto, Close, & Nicotra, 2017), 10 °C for five species in
559 Panama (Rey-Sánchez *et al*., 2016) and in the Amazon (Doughty & Goulden, 2008), 7
560 °C for three species in tropical China (Dong, Prentice, Harrison, Song, & Zhang,
561 2017)). This could be due to high sampling frequency used in this study (every 10 s)
562 compared to others (every 2 minutes in Rey-Sánchez *et al*., 2016, half hourly in Dong
563 *et al*., 2017 and unspecified in Leigh *et al*., 2017) as at high frequency extreme values
564 are more likely to be recorded, though Doughty & Goulden (2008) used a 1 s
565 sampling frequency. It could also be due to the light conditions and study species
566 measured (discussed below). The highest T_L observed (37.2 °C) is less than those
567 reported by others under higher ambient air temperatures (e.g. 45 °C - Doughty &
568 Goulden, 2008; 48 °C – Slot *et al*., 2016, Krause *et al*., 2010). This work supports the
569 view that ambient air temperatures cannot necessarily be used as a proxy for leaf
570 temperature in physiological models as they are not necessarily equal (Michaletz *et*

571 *al*., 2016; Rey-Sánchez *et al*., 2016), and that vegetation models should be tested for
572 their ability to reproduce patterns of ΔT (Dong *et al*., 2017).
573
574 The distributions of T_L and ΔT collected over the ten-day period were significantly
575 skewed (Supplementary Fig. S7, S8, Table 2). This is because under the predominant
576 microclimatic conditions of relatively low PAR and D, ΔT is low (< 1 °C) and T_L is
577 similar to T_A. However, due to fluctuating conditions – especially PAR which alters
578 rapidly with cloud movements and wind, and varies with sun angle, leaf angle and
579 orientation - large increases in ΔT occur. The duration of high ΔT excursions depends
580 on how long the microclimate is sustained. The extent of high ΔT excursions is
581 important because during high leaf temperatures beyond the photosynthetic
582 temperature optima primary productivity will reduce carbon gain and very high leaf
583 temperatures can cause irreversible thermal damage (e.g. above 50 – 53 °C for a
584 Panamanian tree species, Krause *et al*., 2010). Our data suggest that, at least during
585 our measurement period, tree leaves at this Atlantic forest site are not approaching
586 thresholds of irreversible damage, but do reach temperatures known to affect
587 thylakoid membrane structure (35 °C, Gounaris *et al*., 1983; Gounaris *et al*., 1984)
588 and reduce electron transport rates (40 °C, Allakhverdiev *et al*., 2008) although
589 critical temperatures of PSII activity are known to vary among species (O'Sullivan *et*
590 *al*., 2017; Sastry & Barua, 2017). While we do not know the photosynthetic
591 temperature optima of these trees, it is likely that the higher leaf temperatures reached
592 were supra-optimal for photosynthesis despite the low air temperatures.
593
594 Within the range of conditions during the study period, radiation was the most
595 important microclimate variable for determining ΔT (Fig. 9, Supplementary Fig. S10-

596 S12). This has also been shown in other studies (e.g. Doughty & Goulden, 2008; Rey-
597 Sánchez *et al*., 2016) and is understood biophysically (Jones, 1992). For absolute T_L,
598 PAR, T_A, and D were all important (Supplementary Fig. S13-S16), though the
599 strength of the D effect is likely due at least in part to its co-variation with T_A. Recent
600 work has shown the occurrence of a 'crossover' T_A at 25-28 °C (Michaletz *et al*.,
601 2016, Dong *et al*., 2017). The crossover temperature is the T_A at which ΔT=0 and
602 above which ΔT is negative. We found no evidence of a crossover T_A, likely due to
603 the relatively low T_A during our study. The light levels observed in the study were
604 high, occasionally exceeding 3000 μmol m^{-2} s^{-1}. We consider the light levels recorded
605 in the profile to be accurate as they were highly comparable to an independent dataset
606 from a weather station mounted at 27 m on the same tower (Figure S18). The values
607 are higher than the PAR observed in similar studies from lowland forests with typical
608 maximum PAR of 2000 μmol m^{-2} s^{-1} (Rey-Sánchez et al., 2016; Doughty & Goulden,
609 2008). Again, measurement frequency may be important here for recording extreme
610 instantaneous values. In fact, this maximum quantity of PAR is equivalent to more
611 radiation than the solar constant (incoming light at the top of the atmosphere, 1.353
612 kW m^{-2}) which is possible in mountains when light is reflected from clouds
613 (Stoutjesdijk & Barkman, 2014). Incoming radiation increases by 8 % for every 1000
614 m increase in elevation (Blumthaler, Ambach, & Ellinger, 1997). Montane forests are
615 therefore likely to experience higher maximum radiation loads than lowland forest, as
616 has been measured at this site (Rosado, Joly, Burgess, Oliveira, & Aidar, 2016).
617 Given the importance of radiation for T_L, trees at high elevation may have greater risk
618 of hitting damaging T_L thresholds if air temperatures increase with climate change. At
619 this specific site, in additional to increased radiation, D also increases with elevation
620 and trees show more conservative water use (Rosado *et al*., 2016) which will further

influence leaf temperatures. Mountaintop species are already considered to be more greatly threatened than lowland species by increased temperatures as there is no cooler place for species to move to. The high radiation load increasing leaf temperatures may exacerbate this problem.

As microclimate is a key driver of leaf temperature, it is important to consider the vertical gradient in microclimate (Supplementary Figure S6). We found that all microclimate variables displayed vertical gradients, especially during sunny days when the differences between the top and bottom of the canopy exceeded 5 °C T_A, 2200 μmol m^{-2} s^{-1} *PAR* and 1.3 kPa *D*. The difference in T_A leads to a larger difference in T_L than the values of ΔT we typically found (Table 1). Whilst vertical gradients of *PAR* are often accounted for in vegetation models, often the gradients of other key variables are not considered, which would lead to error in quantification of leaf temperatures below the canopy top.

Differences in leaf thermoregulation between species

We found striking differences in leaf temperature patterns between species that were attributable to differing leaf traits. *Miconia* leaves more commonly experienced high ΔT excursions than *Alchornea*, with higher skew in T_L and ΔT distributions, higher maximum ΔT and lower minimum ΔT (Table 1). Leaf temperatures of *Miconia* were consistently higher than *Alchornea* when controlling for microclimate between measurements, and significantly so during high light conditions (Fig. 4). The differences increased with increasing thermal stress (higher PAR, T_A and *D*). The lack of significance at the highest PAR/T_A subset tested is likely due to low data

availability and higher PAR measurement errors at high PAR. As PAR was not measured directly at the leaf surface it was difficult to ensure correspondence between PAR as measured by the nearest sensor and received at the leaf surface; this is more problematic under direct light conditions where leaf angle, orientation, sun angle and within canopy shading greatly impact leaf surface PAR. We recommend all studies of leaf temperature attempt to measure PAR at the leaf surface despite the higher efforts required.

The higher leaf temperatures displayed by *Miconia* can be accounted for by lower transpirational cooling due to two reasons. Firstly, the wider leaf width increases boundary layer resistance, which lowers the evaporation from stomatal pores. Secondly, *Miconia* leaves showed a strong negative relationship between g_s and D which lowers transpiration under conditions of high thermal stress (as high D typically occurs concurrently with high PAR and T_A). Using the leaf energy balance equation we find that the physical difference in leaf width is the dominant factor in producing the variation in ΔT between *Miconia* and *Alchornea* (Supplementary Figure. 17). *Miconia* leaves get hotter than *Alchornea* leaves and hence may have a higher risk of thermal damage. However, this increased heating may come with a water-use advantage, as, under high D conditions, transpiration rates per leaf area will be lower for *Miconia* than *Alchornea*. This could reduce the risk of xylem cavitation under water stress conditions. Differing thermoregulation strategies of trees likely arise in combination with trade-offs in terms of water use.

The study species only showed differing relationships between PAR, and T_L and ΔT, with similar responses to other microclimatic variables (Fig. 9, Supplementary

671 Figures S10-S16). This shows that it is the consequences for input solar energy that
672 varies between the species, rather than differing mechanisms in response to T_A. It is
673 not to say that other microclimatic variables are not important for T_L or ΔT, but that
674 the response of T_L and ΔT to other variables is the same for the two species, at least
675 under the measurement conditions.
676
677 Night-time ΔT were consistently negative for all species. However, ΔT of *Alchornea*
678 leaves were more negative than the other species (Table 1). The cause may be that
679 many of the sampled *Alchornea* leaves were at the outer canopy and therefore heat
680 radiation to space may be more effective for them due to the lack of obstacles (other
681 leaves or canopies), resulting in greater cooling. Another factor may be that
682 transpiration is maintained at night in this species more so than *Miconia* and *Guapira*.
683 Observations from Rosado *et al*. (2012) do show night-time transpiration occurring
684 for *Alchornea* trees at this site, but *Alchornea* did not show higher transpiration than
685 other measured species.
686
687 Leaf temperatures of the subcanopy *Guapira* tree were consistently similar to air
688 temperatures and showed little variation (Table 1) likely due to the canopy position
689 receiving very little light (Fig. 1). However, when the data were subsetted for low
690 PAR conditions only, leaves of *Guapira* still showed a lower ΔT than the two other
691 species (Fig. 4a). This could be due to the narrower leaf width of *Guapira* leaves (Fig.
692 6), though the width is not significantly different from *Alchornea*. It could also be due
693 to the unusual leaf angles displayed by the *Guapira* leaves that were hanging near
694 vertically (Table S1, Figure 6e) which would limit the amount of light received and
695 result in over estimates of the light environment from using a horizontally orientated

696 sensor. Another potential contributor is the long τ values estimated, as T_L is expected
697 to vary less when τ is long (Ball, Cowan, & Farquhar, 1988). The long τ for *Guapira*
698 leaves were a result of the combined low g_s and low LDMC (Fig. 7, Supplementary
699 Figure S9); because water has a higher specific heat capacity than dry leaf matter, the
700 higher water content of *Guapira* leaves causes a longer τ (Vogel, 2009).
701
702 *Towards a better understanding of tropical leaf temperature behaviour*
703
704 The link between functional traits and leaf thermoregulation has been highlighted in
705 recent work (Michaletz *et al*., 2015; 2016). Here, we provide field-based evidence for
706 this link in the most detailed study of leaf energy balance in tropical montane forests
707 to date, and include variation in water-use as a key component. The traits that we find
708 important (leaf width, g_s at high D, LDMC) may possibly connect other axes of plant
709 functional variation (Reich, 2014) – the leaf economics spectrum (Wright *et al*., 2004)
710 and plant hydraulics. Species that are able to maintain transpiration under high
711 thermal stress conditions (high T_A, PAR, D) will require water to supply the
712 transpiration stream from an efficient hydraulic system or from high water
713 capacitance to avoid hydraulic failure. Avoiding extremes of T_L and maintaining open
714 stomata will then have the benefit of keeping T_L closer to the temperature optima of
715 photosynthesis, maintaining a CO_2 supply, and all this while PAR is high to drive a
716 high photosynthetic rate (Ball *et al*., 1988). Conversely, lower transpiration under
717 high thermal stress conditions will prevent excessive water loss and therefore avoid
718 risk of hydraulic failure through xylem embolism, but increase risk of the leaf
719 reaching a damaging high temperature threshold. Critical thresholds of photosynthetic
720 activity vary by species (O'Sullivan et al., 2017). A recent study of critical thresholds

721 of 41 co-occurring tropical species found that variation was related to the leaf
722 economics spectrum (Wright *et al*., 2004), with high LMA species showing higher
723 temperature tolerance (Sastry & Barau, 2017). *Miconia* has significantly higher LMA
724 than *Alchornea* (Fig. 6), and it would be parsimonious if it also displays a higher
725 critical temperature for damage to photosynthetic machinery. In summary, we
726 hypothesise that trees at the 'slow' end of the life-history spectrum (Reich, 2014) are
727 likely to reach higher leaf temperatures, have lower g_s and photosynthesis under high
728 thermal stress conditions, lower risk of hydraulic failure, and a higher threshold for
729 thermal damage, with the converse true of 'fast' species.
730
731 If we are to understand the implications of climate change for tropical forests it will
732 be crucial to understand mechanisms of leaf thermoregulation and how this varies
733 between species. We have based our findings on only a small, if detailed, dataset.
734 There are very few comparable datasets available for tropical forests. More datasets
735 exploring the full energy balance of tropical leaves from multiple sites with varying
736 climatologies, and ideally over extended time periods, would certainly aid this.
737 Beyond understanding current patterns of leaf temperatures, it is also necessary to
738 understand the response of energy balance parameters to high T_A and CO_2. For
739 example, herbarium data for an Australian shrub species showed a reduction in leaf
740 width over the last century (Guerin, Wen, & Lowe, 2012) which could mitigate
741 increases in T_L due to increased T_A. Conversely, declines in g_s are a common response
742 of tree species to increased CO_2 which, while potentially reducing water-use, could
743 lead to higher T_L (e.g. Barker *et al*., 2005; Warren *et al*., 2011). However, the extent
744 of reductions in g_s under elevated CO_2 varies with species (Way et al., 2015). In a
745 study of seedlings of 10 tropical species, Cernusak et al. (2011) found reductions in g_s

746 in all species in response to elevated CO_2, but the reductions were larger for species
747 with high g_s in ambient conditions. Warming may also cause changes in g_s; results
748 from warming experiments show a variety of responses – increases, decreases, no
749 change (Way *et al*. 2015) - and a recent meta-analysis found decreases in stomatal
750 density with higher T_A in trees but not in herbs (Yan, Zhong, & Shangguan, 2017). If
751 trees do indeed decrease g_s under higher growth temperatures this could result in
752 further leaf warming beyond T_A increases, but only if transpiration declines as well as
753 g_s, which is not certain given the expected rise in D with increased T_A. Our
754 understanding of the effects of combined CO_2 and warming is even more limited. If
755 both cause a decline in g_s separately, would the combined effect be additive leading to
756 even greater reductions? The limited experimental data does not paint a clear picture
757 (Way *et al*., 2015). A final question is whether leaves that reach higher temperatures
758 are better adapted to cope with high temperatures and therefore increasing T_L would
759 be less consequential than for low temperature species, or does the fact that leaf
760 temperatures are already high mean that high-temperature species are more at risk?
761
762 *Conclusions*
763 In this study we made detailed measurements of leaf energy balance for three tree
764 species in the montane Atlantic forest, Brazil. Our results show surprising high leaf-
765 to-air temperature differences given the relatively low air temperatures, which we
766 attribute to the high light conditions during the study. The higher radiation levels
767 occurring at high elevations may contribute to the risks of climate change to tropical
768 montane forests. We find differences in leaf thermoregulation between leaves of
769 *Alchornea* and *Miconia*, which is attributable to lower transpiration under high
770 thermal stress conditions for *Miconia* due to its wider leaves and stronger reduction of

771 g_s with increasing *D*. Leaf energy balance modelling can be a powerful tool to
772 understand variation between species in leaf thermoregulation which will be
773 necessary to model the impact of climate change on leaf physiology.
774
775 **Acknowledgements**
776 This research was co-supported by the Natural Environment Research Council/NERC
777 (NE/K016431/1 and NE/N012542/1) and the State of São Paulo Research
778 Foundation/FAPESP (2012/51509-8, 2012/51872-5 and 2014/13322-9) as part of the
779 projects ECOFOR and BIORED, by the Brazilian National Research Council/CNPq
780 (PELD Process 403710/2012-0) within the BIOTA/FAPESP Program - The
781 Biodiversity Virtual Institute, www.biota.org.br, and by the European Research
782 Council project GEM-TRAIT awarded to Yadvinder Malhi. Research was performed
783 with permit COTEC/IF 230/2015. OLP and MJP were funded by the European
784 Research Council project T-Forces (291585). We acknowledge the help of David
785 Ashley and Santiago Clerici for sensor design and production, Rakesh Tiwari for
786 assistance with Figure 1, Emilia Brasilio for providing wind speed data, Wagner
787 Toledo for assistance in the field, and the Serra do Mar State Park for access to the
788 study site.
789
790 **References**
791
792 Allakhverdiev S.I., Kreslavski V.D., Klimov V.V., Los D.A., Carpentier R. &
793 Mohanty P. (2008) Heat stress: an overview of molecular responses in
794 photosynthesis. *Photosynthesis Reviews*, 98, 541-550.
795

796 Ball M.C., Cowan I.R. & Farquhar G.D. (1988) Maintenance of leaf temperature and
797 the optimisation of carbon gain in relation to water loss in a tropical
798 mangrove forest. *Australian Journal of Plant Physiology*, 15, 263-276.
799
800 Barker D.H., Loveys B.R., Egerton J.J.G., Gorton H., Williams W.E. & Ball, M.C.
801 (2005) CO_2 enrichment predisposes foliage of a eucalypt to freezing injury
802 and reduces spring growth. *Plant, Cell and Environment*, 28, 1506-1515.
803
804 Bartoń K. (2016) Package 'MuMIn'. https://cran.r-
805 project.org/web/packages/MuMIn/MuMIn.pdf
806
807 Blumthaler M., Ambach W. & Ellinger R. (1997) Increase in solar UV radiation with
808 altitude. *Journal of Photochemistry and Photobiology B*, 39, 130-134.
809
810 Campbell G.S. & Norman J.M. (1998) An Introduction to Environmental Biophysics.
811 Springer-Verlag, New York, USA.
812
813 Cernusak L.A., Winter K., Martínez C., Correa E., Aranda J., Garcia M., Jaramillo C.
814 & Turner B.L. (2011) Responses of legume versus nonlegume tropical tree
815 seedlings to elevated CO_2 concentration. *Plant Physiology*, 157, 372-385.
816
817 Collins M., Knutti R., Arblaster J., Dufresne J.-L., Fichefet T., Friedlingstein P., …,
818 Wehner M. (2013) Long-term climate change: Projections, commitments
819 and irreversibility. In: Climate Change 2013: The Physical Science Basis.
820 Contribution of working group I to the Fifth Assessment Report of the

821 Intergovernmental Panel on Climate Change (eds Stocker T.F., Qin D.,
822 Plattner G.-K., Tignor M., Allen S.K., Boschung J., …, Midgley P.M.), pp
823 1029-1136, Cambridge University Press, Cambridge, UK and New York,
824 USA.
825
826 Colombo A.F. & Joly C.A. (2010) Brazilian Atlantic Forest *lato sensu*: the most
827 ancient Brazilian forest, and a biodiversity hotspot, is highly threatened by
828 climate change. *Brazilian Journal of Biology*, 70, 697-708.
829
830 Coumou D. & Robinson A. (2013) Historic and future increase in the global land area
831 affected by monthly heat extremes. *Environmental Research Letters*, 8,
832 034018.
833
834 Dong N., Prentice I.C., Harrison S.P., Song Q.H. & Zhang Y.P. (2017) Biophysical
835 homeostasis of leaf temperature: A neglected process for vegetation and
836 land-surface modelling. *Global Ecology and Biogeography*, 26, 998-1007.
837
838 Doughty C.E. & Goulden M.L. (2008) Are tropical forests near a high temperature
839 threshold? *Journal of Geophysical Research*, 113, G00B07.
840
841 Drake B.G., Gonzàlez-Meler M.A. & Long S.P. (1997) More efficient plants: A
842 consequence of rising atmospheric CO_2? *Annual Reviews Plant Physiology*
843 *and Plant Molecular Biology*, 48, 609-639.
844

Galbraith D.R., Levy P.E., Sitch S., Huntingford C., Cox P., Williams M. & Meir P. (2010) Multiple mechanisms of Amazonian forest biomass losses in three dynamic global vegetation models under climate change. *New Phytologist*, 187, 647-665.

Gounaris K., Brain A.R.R., Quinn P.J. & Willams W.P. (1983) Structural and functional changes associated with heat-induces phase separation of non-bilayer lipids in chloroplast thylakoid membranes. *FEBS Letters*, 153, 47-53.

Gounaris K., Brain A.R.R., Quinn P.J. & Willams W.P. (1984) Structural reorganization of chloroplast thylakoid membranes in response to heat stress. *Biochimica et Biophysica Acta – Bioenergetics*, 776, 198-208.

Guerin G.R., Wen H. & Lowe A.J. (2012) Leaf morphology shift linked to climate change. *Biology Letters*, 8, 882-886.

Guilherme F.A.G., Morellato L.P.C., Assis M.A. (2004) Horizontal and vertical tree community structure in a lowland Atlantic Rain Forest, Southeastern Brazil. *Revista Brasileira de Botanica*, 27, 725-737.

Harris I., Jones P.D., Osborn T.J., Lister D.H. (2013) Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. *International Journal of Climatology*, 34, 623-642.

870 Huntingford C, Zelazowski P, Galbraith D., Mercado L.M., Sitch S., Fisher R., …,
871 Cox P.M. (2013) Simulated resilience of tropical rainforests to CO_2-
872 induced climate change. *Nature Geoscience*, 6, 267-273.
873
874 Jayalakshmy M.S. & Philip J. (2010) Thermophysical properties of plant leaves and
875 their influence on the environment temperature. *International Journal of*
876 *Thermophysics*, 31, 2295-2304.
877
878 Joly C.A., Assis M.A., Bernacci, L.C., Tamashiro J.Y., de Campos M.C.R., Gomes
879 J.A.M.A., …, Belinello R. (2012) Florístics e fitossociologia em parcelas
880 permanentes da Mata Atlântica do sudeste do Brasil ao longo de um
881 gradient altitudinal. *Biota Neotropica*, 12, 123-145.
882
883 Joly C.A., Metzger J.P. & Tabarelli M. (2014) Experiences from the Brazilian
884 Atlantic Forest: ecological findings and conservation initiatives. *New*
885 *Phytologist*, 204, 459-473.
886
887 Jones H.G. (1992) Plants and microclimate. Cambridge University Press, Cambridge,
888 UK.
889
890 Krause G.H., Winter K., Krause B., Jahns P., García M., Aranda J. & Virgo A. (2010)
891 High-temperature tolerance of a tropical tree, Ficus insipida:
892 metholodological reassessment and climate change considerations.
893 *Functional Plant Biology*, 37, 890-900.
894

895 Leigh A., Sevanto S., Close J.D., Nicotra A.B. (2017) The influence of leaf size and
896 shape on leaf thermal dynamics: does theory hold up under natural
897 conditions? *Plant, Cell & Environment*, 40, 237-248.
898
899 Leuning R. (1995) A critical appraisal of a combined stomatal-photosynthesis model
900 for C_3 plants. *Plant, Cell and Environment,* 18, 339-355.
901
902 Lloyd J. & Farquhar G.D. (2008) Effects of rising temperatures and [CO_2] on the
903 physiology of tropical forest trees. *Philosophical Transactions of the Royal*
904 *Society B*, 363, 1811-1817.
905
906 Marchiori N.M., Rocha H.R., Tamashiro J.Y. & Aidar M.P.M. (2016) Tree
907 community composition and aboveground biomass in a secondary Atlantic
908 forest, Serra do Mar state park, São Paulo, Brazil. *CERNE*, 21**,** 501-514.
909
910 Michaletz S.T., Weiser M.D., McDowell N.G., Zhou J., Kaspari M., Helliker B.R. &
911 Enquist B.J. (2015) Plant thermoregulation, energetics, trait-environment
912 interactions, and carbon economics. *Trends in Ecology and Evolution*, 30,
913 714-724.
914
915 Michaletz S.T., Weiser M.D., McDowell N.G., Zhou J., Kaspari M., Helliker B.R. &
916 Enquist B.J. (2016) The energetic and carbon economic origins of leaf
917 thermoregulation. *Nature Plants*, doi:10.1038/nplants.2016.129.
918

919 Myers N., Mittermeier R.A., Mittermeier C.G., da Fonseca G.A.B. & Kent J. (2000)
920 Biodiversity hotspots for conservation priorities. *Nature*, 403, 853-858.
921
922 Nakagawa S. & Schielzeth H. (2013) A general and simple method for obtaining R^2
923 from generalized linear mixed-effects models. *Methods in Ecology and*
924 *Evolution*, 4, 133-142.
925
926 O'Sullivan O.S., Heskel M.A., Reich P.B., Tjoelker M.G., Weerasinghe L.K.,
927 Penillard A., …, Atkin O.K. (2017) Thermal limits of leaf metabolism
928 across biomes. *Global Change Biology*, 23, 209-223.
929
930 Pan Y., Birdsey R.A., Fang J., Houghton R., Kauppi P.E., Kurz W.A., …, Hayes D.
931 (2011) A large and persistent carbon sink in the world's forests. *Science*,
932 333, 988-993.
933
934 Penman H.L. (1948) Natural evaporation from open water, bare soil and grass.
935 *Proceedings of the Royal Society of London A*, 193, 120-145.
936
937 Phillips O.L. (1997) The changing ecology of tropical forests. *Biodiversity and*
938 *Conservation*, 6, 291-311.
939
940 Pinheiro J., Bates D., DebRoy S., Sarkar D. & R Core Team (2017) nlme: Linear and
941 Nonlinear Mixed Effects Models. R package version 3.1-131.
942

943 Rahmstorf S., Foster G. & Cahill N. (2017) Global temperature evolution: recent
944 trends and some pitfalls. *Environmental Research Letters*, 12, 054001.
945
946 Reich P.B. (2014) The world-wide 'fast-slow' plant economics spectrum: a traits
947 manifesto. *Journal of Ecology*, 102, 275-301.
948
949 Rey-Sanchez A.C., Slot M., Posada J.M., Kitajima K. (2016) Spatial and seasonal
950 variation in leaf temperature within the canopy of a tropical forest. *Climate*
951 *Research*, 71, 75-89.
952
953 Rosado B.H.P., Oliveira R.S. & Aidar M.P.M. (2010) Is leaf water repellency related
954 to vapour pressure deficit and crown exposure in tropical forests? *Acta*
955 *Oecologica*, 36, 645-649.
956
957 Rosado B.H.P., Oliveira R.S., Joly C.A., Aidar M.P.M. & Burgess S.O. (2012)
958 Diversity in nighttime transpiration behaviour of woody species of the
959 Atlantic Rain Forest, Brazil. *Agricultural and Forest Meteorology*, 158-
960 159, 13-20.
961
962 Rosado B.H.P., Joly C.A., Burgess S.S.O., Oliveira R.S. & Aidar M.P.M. (2016)
963 Changes in plant functional traits and water use in Atlantic rainforest:
964 evidence of conservative water use in spatio-temporal scales. *Trees*, 30, 47-
965 61.
966

967 Sastry A. & Barua D. (2017) Leaf thermotolerance in tropical trees from a seasonally
968 dry climate varies along the slow-fast resource acquisition spectrum.
969 *Scientific Reports*, 7, 11246.
970
971 Singsaas E.L. & Sharkey T.D. (1998) The regulation of isoprene emission responses
972 to rapid leaf temperature fluctuations. *Plant, Cell and Environment*, 21,
973 1181-1188.
974
975 Slot M., Garcia M.N. & Winter K. (2016) Temperature response of CO_2 exchange in
976 three tropical tree species. *Functional Plant Biology*, 43, 468-478.
977
978 Slot M. & Winter K. (2017) In situ temperature response of photosynthesis of 42 tree
979 and liana species in the canopy of two Panamanian lowland tropical forests
980 with contrasting rainfall regimes. *New Phytologist*, 214, 1103-1117.
981
982 Stoutjesdijk P. & Barkman J.J. (2014) Microclimate, Vegetation & Fauna (2^{nd} Ed.),
983 KNNV Publishing, Netherlands.
984
985 Teskey R., Wertin T., Bauweraerts I., Ameye M., McGuire M.A. & Steppe K. (2015)
986 Responses of tree species to heat waves and extreme heat events. *Plant Cell*
987 *& Environment*, 38, 1699-1712.
988
989 Vialet-Chabrand S.R.M., Matthews J.S.A., McAusland L., Blatt M.R., Griffiths H. &
990 Lawson T. (2017) Temporal dynamics of stomatal behaviour: Modeling

and implications for photosynthesis and water use. *Plant Physiology*, 174, 603-613.

Vieira S.A., Alves F.A., Duarte-Neto P.J., Martins S.C., Veiga L.G., Scaranello M.A., …, Martinelli L.A. (2011) Stocks of carbon and nitrogen and partitioning between above- and belowground pools in the Brazilian coastal Atlantic Forest elevation range. *Ecology and Evolution*, 3, 421, 434.

Vogel S. (2009) Leaves in the lowest and highest winds: temperature, force and shape. *New Phytologist*, 183, 13-26.

Warren J.M., Norby R.J. & Wullschleger S.D. (2011) Elevated CO_2 enhances leaf senescence during extreme drought in a temperate forest. *Tree Physiology*, 31, 117-130.

Way D.A., Oren R. & Kroner Y. (2015) The space-time continuum: the effects of elevated CO_2 and temperature and the importance of scaling. *Plant, Cell and Environment*, 38, 991-1007.

Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., …, Villar R. (2004) The worldwide leaf economics spectrum. *Nature*, 428, 821-827.

Wright I.J., Dong N., Maire V., Prentice I.C., Westoby M., Díaz S., …, Wilf P. (2017) Global climatic drivers of leaf size. *Science*, 357, 917-921.

1016 Yan W., Zhong Y. & Shangguan Z. (2017) Contrasting responses of leaf stomatal
1017 characteristics to climate change: a considerable challenge to predict carbon
1018 and water cycles. *Global Change Biology,* 23, 3781-3793.
1019

1020 **Table 1**. Leaf temperature variables for three species. n = number of leaves measured

1021 for each tree.

	Mean ± SD			
	Alchornea (n=10)	*Miconia* (n=9)	*Guapira* (n=4)	*p**
Daytime minimum T_L (°C)	7.71 ± 0.63	8.51 ± 1.55	8.13 ± 0.59	0.18
Daytime mean T_L (°C)	15.98 ± 0.47	16.14 ± 0.72	14.6 ± 0.01	0.6
Daytime maximum T_L (°C)	30.56 ± 3.6	34.63 ± 2.63	23.33 ± 1.22	0.012
Daytime T_L skewness	0.84 ± 0.43	1.38 ± 0.27	0.56 ± 0.27	0.005
Daytime minimum ΔT (°C)	-3.72 ± 1.36	-2.16 ± 0.75	-5.07 ± 2.50	0.007
Daytime mean ΔT (°C)	0.79 ± 0.40	0.84 ± 0.43	0.007 ± 0.001	0.8
Daytime maximum ΔT (°C)	11.27 ± 4.15	14.23 ± 2.71	3.28 ± 1.08	0.08
Daytime ΔT skewness	2.51 ± 1.32	4.56 ± 1.63	0.73 ± 5.86	0.009
Night-time mean ΔT (°C)	-0.13 ± 0.07	-0.06 ± 0.03	-0.008 ± 0.01	0.02

1022 * p-value from t-tests comparing *Alchornea* and *Miconia.*

1023

Table 2. ANOVA table for the linear mixed effects model of *D*, species, and their interaction on g_s. Leaf is included as random intercept.

	Numerator DF	Denominator DF	F	p
Intercept	1	167	1097.0	< 0.0001
D	1	167	18.7	< 0.0001
Species	2	20	40.0	< 0.0001
D:Species interaction	2	167	6.6	0.0018

Figure legends

Figure 1. Schematic of field data collection showing positions of microclimate measurements and leaves sampled for temperature.

Figure 2. Time-series of microclimate and leaf-to-air temperature difference on 8 October 2016. a) Photosynthetically active radiation, b) air temperature, c) vapour pressure deficit, d) leaf-to-air temperature difference for leaves of *Alchornea* (A6), *Miconia* (M1) and *Guapira* (G3). Colours refer to measurement heights.

Figure 3. Leaf (T_L) and air (T_A) temperatures measured over 10 days for a) Alchornea (10 leaves), b) Miconia (10 leaves) and c) Guapira (4 leaves). Colour denotes leaf height. Grey line – y=x. Each data point is single measure of a single leaf taken from the thermocouple time series recorded every 10 s.

Figure 4. Leaf to air temperature difference (ΔT) variation between species and microclimatic conditions. Vertical lines show the mean value for the species. Significance values are shown testing for differences between species (linear mixed effects model with leaf as a random factor) under four different microclimates. PAR – photosynthetically active radiation, T_A – air temperature.

Figure 5. Relationship between stomatal conductance and vapour pressure deficit, and variation between species. Equations for each species – Alchornea: g_s = 325.1 (± 22.4) – 68.8 (± 22.6) D; Guapira: g_s = 185.4 (± 36.4) – 212.7 (± 22.6) D; Miconia: 401.6 (± 31.0) – 189.8 (± 35.4) D (errors are SE).

Figure 6. Variation in leaf structural traits between species. Letters represent significantly different groups calculated using ANOVA and Tukey post-hoc tests.

Figure 7. Distributions of thermal time constants estimated for each species (a), and the relationship between stomatal conductance and the thermal time constant (b).

Figure 8. Leaf to air temperature difference (ΔT) from observations and energy balance estimations. Grey dash – y=x line, grey solid – linear regression line for all data, regression slope = 0.72 ± 0.03 SE, intercept -0.41 ± 0.07 SE, $F = 725.8$, $df = 185$, $p < 0.0001$, $R^2 = 0.80$.

Figure 9. Relationships between PAR and observed ΔT (a, b) and estimated ΔT_e (c, d) for *Alchornea* (a, c) and *Miconia* (b, d). Solid line – modelled relationship for the plotted species, dash line – modelled relationship for the alternative species. Statistical models are linear mixed effects model with leaf as a random factor. R^2 is the marginal pseudo R^2. To account for uneven sampling with respect to PAR data was subsampled for 1000 points in 250 μmol m^{-2} s^{-1} bins for points below 1000 μmol m^{-2} s^{-1}.